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Anti-inflammatory and analgesic effects of ethanol and aqueous extracts of *Pterocephalus hookeri* (C.B. Clarke) Höeck

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ABSTRACT

Aim of the study: This study evaluates the anti-inflammatory and analgesic activities of the ethanol and aqueous extracts of a Tibetan herb *Pterocephalus hookeri* (C.B. Clarke) Höeck to provide experimental evidence for its traditional use such as cold, flu and rheumatoid arthritis.
Materials and methods: Investigations on the analgesic effects of *P. hookeri* (C.B. Clarke) Höeck were carried out, including hot-plate test and acetic acid-induced writhing. The anti-inflammatory activities were observed by utilizing the following models: carrageenin-induced edema of the hind paw of rats, cotton pellet-induced granuloma formation in rats, acetic acid-induced permeability, and xylene-induced ear edema in mice. The effects of the administration of indomethacin were also studied.
Results: It has been shown that the ethanol and aqueous extracts significantly increased the hot-plate pain threshold and reduced acetic acid-induced writhing response in mice. The ethanol and aqueous extracts remarkably inhibited the increase in vascular permeability induced by acetic acid and ear edema induced by xylene. The ethanol extract also significantly decreased the carrageenin-induced rat paw edema perimeter and inhibited the increase of granuloma weight.
Conclusion: The results show that the ethanol and aqueous extracts have both central and peripheral analgesic activities and as anti-inflammatory effects, supporting the traditional application of this herb in treating various diseases associated with inflammation and pain.

1. Introduction

Traditional Chinese herbal medicines, including Tibetan herbs, are used in the treatment of a wide variety of clinical diseases in China. *Pterocephalus hookeri* is one of the popular Tibetan herbs and the medicinal part is the total leaves of *P. hookeri* (C.B. Clarke) Höeck (family: Dipsacaceae) (Institute of Qinghai Tibetan Medicine and Qinghai Institute for Drug Control, 1996). *P. hookeri*, locally known as "Bang-zi-du-wu" in Tibetan language, is recorded in the typical Tibetan medicine book "Si-Bu-Yi-Dian" (Li, 1983). *P. hookeri* has been widely applied in many Tibetan medicine prescriptions and has multiple traditional uses in the treatment of illnesses such as cold, flu, rheumatoid arthritis, and enteritis in China (Pang, 2007).

Recent phytochemical studies reveal that triterpenoid saponins, songoroside A, 1oganin, palmitic acid, ursolic acid, oleanolic acid, sitostero1, gentiobiose, and rivularici were the principal constituents of *P. hookeri*, all reported to have anti-inflammatory effects (Dai et al., 1989; Tian et al., 1993, 2000, 2002; Zhang et al., 2002). The butanol extract of *P. hookeri* has been found to inhibit inflammation with less toxicity (Guan et al., 2004). All the traditional uses are most likely related to the herb's anti-inflammatory and analgesic actions, and yet few reports on the pharmacological properties of the aqueous and ethanol extracts of *P. hookeri* are available. Thus it is worthwhile to evaluate its analgesic and anti-inflammatory activities in rats and mice.

In this study, the authors examined the effects of aqueous and ethanol extracts of *P. hookeri* on hot-plate, acetic acid-induced writhing, vascular permeability, xylene-induced ear edema, carrageenin-induced edema of the hind paw, and cotton pellet-induced formation of the granuloma tests in rats and mice.

2. Materials and methods

2.1. Plant materials

The leaves of *Herba P. hookeri* (C.B. Clarke) Höeck, which were provided by the Science and Technology Department of Lasa City, Xizang Autonomous Region, were collected in July 2003. The plant identified as *P. hookeri* (C.B. Clarke) Höeck was confirmed by

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Professor Weisuo Fang of the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China. The voucher specimen (No. NCPS006) was deposited in the laboratory for further reference.

2.2. Chemicals

Indomethacin capsule was obtained from Hebei Yongfeng Limited Company (Hebei, China). Acetic acid and xylene were purchased from Beijing Chemical Company (Beijing, China). Carrageenin was obtained from Sigma (St. Louis, MO, USA). Evans Blue was obtained from Shanghai Chemical Company (Shanghai, China).

2.3. Preparation of plant extracts

The dried leaves of *P. hookeri* were ground into fine powder in a blender and extracted using 95% ethanol for 3 h, 2 h and 2 h. Ethanol extract solutions were mixed. The residue of the plant was extracted using distilled water for 1.5 h. The ethanol and aqueous extracts were filtered and concentrated in a rotor evaporator, and then dried in an oven (50 °C). The yields of ethanol and aqueous extracts were 42.25% and 9.01%, respectively. The extracts were stored in a refrigerator for 4 °C, and dilutions of the extracts were performed using distilled water before determining their effects.

2.4. Animals

Male or female Imprinting Control Region (ICR) mice (20–22 g) and male Sprague–Dawley rats (180–200 g) were purchased from the Institute of Experimental Animals of the Chinese Academy of Medical Sciences. Animal welfare and experimental procedures complied with China regulations, specifically the approved protocols of the Animal Ethics Committee of the Chinese Academy of Medicine Sciences, China. All animals were kept in a room maintained under environmentally controlled conditions of 24 ± 1 °C and 12 h light–12 h dark cycle. All animals had free access to water and standard diet. They were acclimatized at least 1 week before the experiments were started. The mice were fasted for 10 h prior to the experiments, and the test substances were given orally with free access to water.

2.5. Drug administration

The ethanol extract (1 g/kg and 2 g/kg), aqueous extract (2 g/kg and 4 g/kg), and indomethacin (10 mg/kg, reference drug) were given orally to mice and rat. The primary experiments showed that these doses selected are suitable for the study. The control group received the same volume of distilled water.

2.6. Writhing reflex induced by acetic acid in mice

In the writhing test, male mice were divided into six groups. This was performed according to the method previously described (Gaertner et al., 1999). The mice received 0.6% acetic acid solution in normal saline injected intraperitoneally at a dose of 10 ml/kg. The number of writhes was counted starting 5 min after injection that lasted for 15 min. The response consisted of abdominal wall contractions, pelvic rotation, followed by hind limb stretches. The test samples, indomethacin, and distilled water were administered orally 1 h prior to acetic acid injection. The percentage analgesic activity was calculated as follows:

$$\text{Percentage analgesic activity} = \frac{Nc - Nt}{Nc} \times 100\%$$

where *Nc* is the average number of stretches of the control group, and *Nt* is the average number of stretches of the test drug group.

2.7. Hot-plate test

This test was performed according to the method previously reported (Lanhers et al., 1991). The hot-plate test was carried out at a fixed temperature of 55 ± 0.5 °C. The reaction consisted of paw licking and jumping. The time in seconds between the platform and reaction was recorded as the response latency. The mice exhibiting latency time greater than 30 s or less than 5 s were excluded. The latency time was determined at 30 min, 60 min, and 90 min after administration of the test samples, indomethacin, and distilled water. If the reaction time was more than 60 s, the latency was recorded as 60 s.

2.8. Ear edema induced by xylene in mice

The xylene-induced ear edema test was previously described (Kou et al., 2005). A total of 30 μl of xylene was given on the anterior and posterior surfaces of the right ear lobe. The test samples, indomethacin, and the distilled water were administered orally 1 h prior to giving xylene. One hour later, the animals were sacrificed by cervical dislocation, and the right and left ears of each animal were removed. The left ear was considered as control. Circular sections were taken with a cork borer (diameter of 7 mm) and weighed.

2.9. Peritoneal permeability induced by acetic acid in mice

The test of peritoneal permeability induced by acetic acid was carried out according to a modified method described in an earlier study (Whittle, 1964). The mice were intravenously injected with 10 ml/kg of 1% Evans Blue dye solution in saline, followed by intraperitoneal injection of 10 ml/kg of 0.7% acetic acid. The ethanol extract (1 g/kg and 2 g/kg), aqueous extract (2 g/kg and 4 g/kg), indomethacin (10 mg/kg), and distilled water were administered orally 1 h prior to the injection of Evans Blue. Twenty minutes after injection of acetic acid, the mice were sacrificed by cervical dislocation. Peritoneal fluids were collected by washing with 5 ml of normal saline, and then centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was read at 590 nm using a spectrophotometer (M5, MD Company, USA).

2.10. Rat paw edema induced by carrageenin

The test of carrageenin-induced rat paw edema was done using a previously reported technique (Winter et al., 1962). Approximately 50 ml of a 1% suspension of carrageenin in saline was prepared 1 h before the test. A total of 50 μl of carrageenin was injected intradermally into the plantar surface of the right hind paw of rats. The test samples, indomethacin, and distilled water were administered orally 1 h prior to injection of carrageenin. The edema diameter was determined at 0 h, 1 h, 2 h, 3 h, and 4 h after carrageenin injection. Measurement of paw size was carried out by wrapping a piece of cotton thread around the paw, and the length of the thread corresponding to the paw circumference was determined using a meter rule. The average perimeter (cm) of the right hind paw of each rat was calculated from three readings.

2.11. Granuloma formation induced by cotton pellet in rats

The test for cotton pellet-induced granuloma formation was described in a previous study (Swingle and Shideman, 1972). The test samples, indomethacin, and distilled water were administered orally once daily for 7 days. A sterilized cotton pellet weighing 20 mg was put subcutaneously into the groin region of rats on the first day. The animals were sacrificed on the eighth day. The pellets, which by then were surrounded by granuloma tissue (seven animals per